968-24 Genetic Modification of Atherosclerotic Arteries Following Balloon Angioplasty

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Genetic modification of the vessel wall by catheter-based techniques offers great potential in the treatment of atherosclerotic vascular disease. This study was therefore undertaken to characterize the response of atherosclerotic vessels exposed to adenoviral vectors following balloon angioplasty. Atherosclerotic lesions were created in iliac arteries of NZW rabbits by endothelial denudation. After 4–6 weeks on a 1.5% cholesterol diet, balloon angioplasty was performed at the site of maximal stenosis detected by iliac angiography. Recombinant adenovirus encoding a nuclear-localizing variant of E. coli β-galactosidase (βGal) was then delivered to the vessels by 1 of 4 techniques: a double balloon catheter (n = 7), a Hydrogel-coated balloon catheter (n = 3), a perforated balloon infusion catheter (n = 3), or a Dispatch catheter (n = 4). Four days after infection, sections of the modified vessels were harvested and stained with X-gal solution. Eosin-counterstained sections were examined for the presence and location of βGal activity. Successful gene transfer was observed in all sections displaying evidence of balloon-induced barotrauma, manifested primarily by arterial dissection, regardless of the device used. Genetically modified cells were located in pockets within the intima, media, and adventitia bordering dissection planes, or in areas adjacent to defects in the internal elastic membrane. To a lesser degree, cells expressing βGal were found in periluminal regions of the intact intima. Extensive transmural or circumferential diffusion of the vector was not seen.

In conclusion, gene transfer to an atherosclerotic vessel subjected to balloon angioplasty is feasible with recombinant adenovirus vectors and currently available delivery catheters. The regions of the vessel wall which express the foreign protein are those which contribute to the proliferative cellular response which characterizes post-angioplasty restenosis.

968-25 In Vivo Incorporation Kinetics of ^{35}S Labelled Antisense *c-myc* Oligonucleotides in Rabbits Carotid Arteries

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Antisense oligonucleotides oligonucleotides complementary to *c-myc* mRNA inhibit smooth muscle cell proliferation *in vitro* and may reduce the proliferative response to vascular injury *in vivo* following balloon angioplasty. The purpose of this study was to evaluate the *in vivo* incorporation kinetics of ^{35}S labelled phosphorothioate antisense c-myc oligonucleotides by assessing the effect of transfection time, of lipofectin or lipofectamin on tissue penetration and retention following transfection in the carotid artery. A total of 117 New Zealand white rabbit carotid arteries were transfected with 80 μM of *c-myc* antisense oligonucleotide in a 1 cm portion either alone (n = 37) or in combination with lipofectin (100 μg/ml or 500 μg/ml) (n = 50) or lipofectamin (100 μg/ml or 500 μg/ml) (n = 30) for periods of either 15, 30, 60, 120 or 240 minutes. Radiolabelled oligonucleotide incorporation level was evaluated by automated scintillation counting and tissue localisation by dot counting following radiographic emulsion exposure of histological sections. Retention evaluation was performed at 24, 48 and 72 hours post-transfection. A high correlation factor ($r^2 = 0.96$) was obtained between the two evaluation methods. Maximal incorporation was seen after 15 min exposure (7.84% ± 1.59%, $p < 0.05$ vs other groups). Maximal radioactivity retained was found at 24 hours (0.743% incorporation ± 0.096%), decreasing rapidly thereafter (0.57% at 48 hours and 0.247% at 72 hours, $p < 0.05$). Efficiency of transfection was not increased by lipofectin or lipofectamin. Labelled oligonucleotide were located mainly in the media (3.85E + 07 dots ± 5.85E + 06 dots) and significantly less in the adventia (7.55E + 06 dots ± 1.84E + 06 dots). *Conclusions:* 1-*In vivo* vascular transfection of phosphorothioate oligonucleotides, without specific vectors, results in good medial tissue penetration, with however a low retention level after 24 hours. 2- This transfer modality does not appear to be enhaced with the use of liposomes.

968-26 In Vitro Antiproliferative Effect of Antisense Oligonucleotides: Are the Effects Specific and Reproducible?

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The involvement of proto-oncogenes in cell proliferation is well documented. One of these, the oncogene *c-myb* is a DNA-binding protein that regulates cell growth and differentiation. In-vitro studies have shown marked inhibition of smooth muscle cell (SMC) proliferation using antisense (AS) *c-myb* oligonucleotides. The purpose of this study was to determine whether the in-vitro antiproliferative effect of AS oligonucleotides is specific and reproducible. Rat aortic SMC were plated at a 1:3 split ratio in 1% fetal bovine serum (FBS), and made quiescent 24 hr later by changing the medium to 1% control process serum replacement II. Cells were stimulated with PDGF (2 ng/ml), and at the same time, oligonucleotides were added. Cell proliferation was determined by measuring ^3H-Thymidine uptake at 18–22 hr. To compare the specificity of the antiproliferative effects, 20 μM of AS *c-myb* and several control oligos were used. These included (Batch A): 18-mer sense (S) c-myb, 19-mer mouse antisense and four-base mismatch AS to IL-1β, and 18- and 16-mer human AS to CML. To determine the reproducibility of the AS effect, the same 18-mer AS *c-myb* and scrambled (Sc) 18-mer from another source (B) were used under exactly the same conditions. To further evaluate the reproducibility of the AS effect, two different preparations (Batch C) of the same 18-mer AS and S *c-myb* from the initial manufacturer were tested under exactly the same culture conditions.

Conclusions: This study shows: 1) that antisense oligonucleotides produce a non-specific in-vitro antiproliferative effect and 2) the in-vitro results may vary with respect to source and batches of oligonucleotides. The lack of consistency in results raises significant questions about clinical therapeutic potential.

968-27 Basic Fibroblast Growth Factor has Potent Selective Effects on Collagen Gene Expression by Human Vascular Smooth Muscle Cells

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Basic fibroblast growth factor (bFGF) is a smooth muscle cell (SMC) mitogen known to play a role in the early response of the vessel wall to balloon injury. During this initial stage, remodeling of the extracellular matrix (ECM), including collagen, is necessary for cell proliferation, migration and lesion expansion. We therefore determined the effect of bFGF on fibrillar collagen gene expression by human vascular SMCs.

SMCs, cultivated from internal mammary artery, were serum-deprived and incubated with bFGF (50 ng/ml) or vehicle. Collagen synthesis rate was determined by labeling cultures with [^3H]proline, separating proteins by electrophoresis, and visualizing and quantifying bands by fluorography and laser densitometry, respectively. This revealed a significant, net decrease in collagen synthesis induced by bFGF. To determine the mechanism of this response, the effect of bFGF on collagen mRNA was determined by Northern blot analysis using probes specific for α-chains of collagen types I, III, and V, accounting for the major and minor fibrillar collagens found in human atherosclerotic plaque. Cell proliferation was assessed by probing for histone 3.2. Despite a consistent increase in SMC proliferation, bFGF *reduced* expression of α_1(I) collagen mRNA by up to 90%. In contrast, bFGF *increased* expression of α_1(III) and α_2(V) collagen mRNA by approximately 20%. Because type III and V collagen fibers are finer than type I, the divergent results may reflect a shift to a less mature collagen lattice induced by bFGF.

Conclusion: Total fibrillar collagen synthesis by human SMCs is reduced by bFGF, due to a potent selective inhibition of type I collagen expression. This effect of bFGF on the ECM may be a prerequisite for SMC accumulation and lesion formation following balloon angioplasty.

968-28 Sexual Dimorphism of Myointimal Proliferation After Balloon Injury of Rat Carotid Artery is Estrogen Dependent

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Atherosclerotic vascular disease progresses more slowly in females with functional ovaries than in males. Balloon injury of the rodent carotid artery is widely used as a model for the early injury phase of atherosclerosis. The current study tested whether female Sprague-Dawley rats (SD) have a reduced (compared to males) vascular growth response to balloon injury and whether this response is estrogen dependent. 10 wk old ♂ and ♀ SD rats were either gonadectomized or studied intact. Gonadectomized rats of both sexes were implanted with (1) 15 mm (empty), (2) 10 mm (filled with 17-β estradiol [E]) or (3) 15 mm (filled with testosterone [T]) silastic capsules 3 days before balloon injury of the carotid artery. Two wks later, rats were perfused with 10% formalin at 120 mmHg and carotid arteries were fixed and subjected to morphometric analysis. Intima/media ratios (I/M%) expressed as degree of myointimal proliferative response to vascular injury were (means ± SEM):

	♂		+ E	+ T
I/M%	132 ± 6 (11)	132 ± 12 (6)	65 ± 11 (6)*	117 ± 19 (7)
	♀		+ E	+ T
I/M%	82 ± 10 (11)#	133 ± 25 (6)*	38 ± 8 (5)*#	123 ± 29 (5)

*$p < 0.05$ compared to their respective intact controls, #$p < 0.05$ compared to their respective male groups

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